IL-37 inhibits lipopolysaccharide-induced osteoclast formation and bone resorption in vivo

著者	JAFARI SAEED
number	44
学位授与機関	Tohoku University
学位授与番号	歯博第748号
URL	http://hdl.handle.net/10097/00097027

氏　名（本籍）	：	JAFARI（ジャファリ） SAEED（サイード）	
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論文審査委員	：	（主査）教授　齋　藤　正　寛 教授　高　田　春比古　　教授　山　本　照　子	

論文内容要旨

IL-37 is a newly defined member of the IL-1 cytokine family. IL-37 expressed only in certain types of human organs and cells such as; testis, thymus, uterus, kidney, heart, peripheral blood mononuclear cells (PBMCs) and dendritic cells. It has been reported that IL-37 inhibited innate immunity and inflammatory responses in autoimmune diseases and tumors. Osteoclast recruitment might be central to diseases involving bone erosion, such as rheumatoid arthritis. Osteoclasts derived from bone marrow cells regulate bone resorption and remodeling. Two factors are required for osteoclasts formation and activation: receptor activator of NF-kB ligand (RANKL) and macrophage colony stimulating factor (M-CSF). It has also been reported that tumor necrosis factor (TNF)- α induced osteoclast formation *in vitro* and *in vivo*. Lipopolysaccharide (LPS), which is a bacterial cell wall component, is known as a potent inducer of inflammation. LPS is able to induce osteoclast formation and pathological bone resorption. LPS is also recognized to induce the production of many local factors, including proinflammatory cytokine such as TNF-α and IL-1, from macrophages or other cells in inflammatory site. These cytokines have been reported to associate with LPS-induced osteoclast formation and bone destruction *in vivo* and *in vitro* studies. Moreover, it has been reported that LPS stimulates osteoblasts to produce or secrete RANKL. IL-37 also inhibited Lipopolysaccharide (LPS)-induced immunological reaction. However, there is no study to investigate the effect of IL-37 on LPS-induced osteoclast formation and bone resorption. The purpose of this study is to investigate the effect of IL-37 in LPS-induced osteoclast formation and bone resorption.

LPS was administrated with or without IL-37 by subcutaneous injection on supracalvariae of mice. The number of osteoclasts, the level of mRNA for cathepsin K and tartrate-resistant acid

phosphatase (TRAP), and the level of C-Terminal Telopeptide Fragments of Type I Collagen Cross-Links as a marker of bone resorption in mice administrated both LPS and IL-37 were lower than that in mice administrated LPS alone. Mice calvariae were observed using microfocal computed tomography images, many bone destruction spots were seen in the group administered LPS. The ratio of the bone destruction area in the LPS administered group was significantly higher than that in the PBS group and IL-37 alone administrated group. In addition, in the LPS and IL-37 administered groups, bone destruction was lower than that in the LPS alone administered group.

Real-time RT-PCR was performed to analyze osteoclast related cytokines RANKL, TNF-α and IL-1β mRNA levels *in vivo*. RANKL, TNF-α and IL-1β mRNA was increased in the LPS administrated mice as compared with PBS administrated groups. On the other hand, RANKL, TNF-α and IL-1β mRNA was inhibited in the IL-37 and LPS administrated mice as compared with LPS alone administrated group.

In vitro analysis, there was no effect of IL-37 in RANKL-induced osteoclast formation, TNF-α-induced osteoclast formation and cell viability from bone marrow macrophages as osteoclast precursor and LPS-induced RANKL expression from stromal cells.

These results indicated that IL-37 inhibited LPS-induced osteoclast formation and bone resorption via inhibition of LPS-induced osteoclast related cytokines, but might not directly inhibit osteoclast formation on osteoclast precursor and RANKL expression on stromal cells.

審査結果要旨

Osteoclast recruitment plays an important role in bone diseases such as rheumatoid arthritis. Osteoclast formation in bone disease is regulated by inflammatory response including bacterial infection and cytokines such as TNF-α, receptor activator of NF-κB ligands (RANKL) and macropharge colony stimulating factor (M-CSF). Lipopolysaccharide (LPS) is a potent inducer of inflammation and pathogen of inflammatory bone loss through the production of inflammatory cytokines including TNF-α, IL-1 and RANKL. Thus inhibition of inflammatory response that involved in osteoclast formation is critical in suppressing bone destruction of rheumatoid arthritis. IL-37 is a new member of the IL-1 cytokine family that expressed certain type of the human organs such as testis, thymus, uterus, kidney, heart, peripheral blood mononuclear cells and dendritic cells. It has been reported that IL-37 inhibited innate immunity and inflammatory responses in autoimmune diseases and tumors. However, no study examined the effect of IL-37 on osteoclast formation induced by inflammatory response.

This study is attempted to investigate effect of IL-37 on LPS-induced osteoclast formation and bone resorption. LPS was administrated with or without IL-37 to supracalvariae of mice. Results indicated that expressions of mRNA for cathepsin K and tartrate-resistant acid phosphatase (TRAP) as markers for osteoclast formation were decreased in LPS and IL-37 administration group compared with LPS alone administration group. Amount of C-terminal telopeptide fragment of type I collagen, a maker of bone destruction was also decreased by administration of IL-37. Microfocal computed tomography analysis demonstrated that spots of bone destruction were significantly decreased in the LPS and IL-37 administration group. The mRNA expressions of osteoclast related cytokines such

as RANKL, IL-1 β and TNF- α were also decreased in the LPS and IL-37 administration group compared with LPS alone administration group. However RANKL induced-osteoclast formation using bone marrow derived cells as osteoclast precursors were not inhibited by the treatment with IL-37.

From these findings, the author concluded that IL-37 inhibited LPS-induced bone resorption through the inhibition of production of osteoclast related mediators such as RANKL, TNF- α and IL-1 β in mice model. However, IL-37 did not inhibit the osteoclastgenesis induced by RANKL or TNF- α in murine bone marrow cells culture. These data indicated that IL-37 inhibited LPS induced bone resorption by inhibiting expression of LPS-induced osteoclast related genes, but it did not directly affect function of osteoclasts.

As the manuscript presented is a very well thought out and very well written, it is suitable to Ph.D thesis.